Report No.: 25,402

25402

CHEMAGRO CORPORATION

Research and Development Department

TITLE: A Gas Chromatographic Method for the Determination of BAY 68138 and Metabolite Residues in Crops

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ABSTRACT: A specific gas chromatographic procedure for the determination of residues of BAY 68138 and its metabolites in various crops involves oxidation to the sulfone with potassium permanganate. Final detection is by thermionic flame ionization. Recovery experiments were run on several crops by adding BAY 68138 or metabolites at the blending step. Recoveries were generally in the 80 - 100% range. An interference study is included along with a confirmatory procedure.

DATE: August 7, 1969

REFERENCES: Notebook References: 68-35; 69-49; 69-77

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Biochemical Methods Manual

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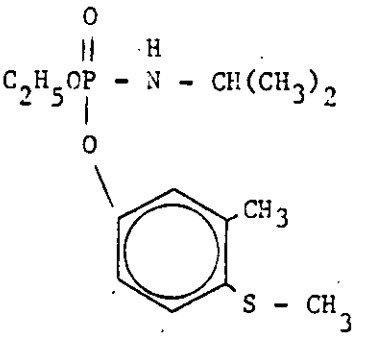
Introduction

BAY 68138 is a nematocide of low volatility which is being developed by Chemagro Corporation. The structural formula is as follows:



The metabolism of BAY 68138 in plants was studied by Waggoner (5) who found that the major metabolite was the sulfoxide. Lesser amounts of the sulfone were also formed.

The residue procedure thus developed is sensitive to the parent compound and its sulfoxide and sulfone. A multicomponent analysis increases the possibility of interference from crop extractives or other pesticides. Therefore, an oxidation was included to convert all the compounds to the sulfone. The sulfone was then measured gas chromatographically, employing a potassium chloride thermionic flame detector.

ANALYTICAL METHOD

Apparatus Required

Assorted laboratory glassware
Blender, Waring or equivalent
Chromatographic column, 20 x 400 mm. with integral 300-ml. reservoir
Food chopper, Hobart or equivalent
Gas chromatograph, Hewlett-Packard Model 5750 equipped with flame ionization detector, or equivalent
Rotary vacuum evaporator, Swissco Model VE-50 or all-glass equivalent
Water bath, 40°C
Wiley Mill

Reagents Required

Acetone, reagent A.C.S., redistilled
Acetone: chloroform mixture 20:80 (V/V)
Acetonitrile, technical, redistilled (Saturate with Skellysolve B before use)

Reagents Required - (Continued)

BAY 68138 standard solution (5 γ/ml in acetone)
- Weigh 0.05 gram of BAY 68138 standard into a clean 100-ml. volumetric flask. Make to volume with reagent acetone and shake to mix.
- Transfer 1 ml. of this solution to a clean 100-ml. volumetric flask. Make to volume with reagent acetone and shake to mix. This flask contains 5 γ/ml. of BAY 68138

Chloroform, reagent, A.C.S., redistilled
Florisil, PR grade 60-100 mesh. Heat in oven at 130°C for 24 hours then deactivate by adding 7% water (7 ml. H_2O + 93 grams of dried Florisil). Mix well. Allow to equilibrate in a tightly stoppered bottle for 24 hours.
Hyflo Super-cel, Johns-Manville
Magnesium sulfate, 20% (W/V) aqueous solution
Methanol, reagent A.C.S., redistilled
Methanol:0.05N Sulfuric acid mixture (60:40 V/V)
Potassium permanganate, 0.1 N aqueous solution
Skellysolve B, redistilled (Saturate with acetonitrile before use)
Sulfuric acid solution, 0.05N in tap water

Extraction Procedure

Citrus peel, citrus pulp, pineapple fruit

1. Grind the entire sample in a food chopper with an equal weight of dry ice. Mix thoroughly. Place the sample in frozen storage overnight to allow the dry ice to sublime.

2. Place 100 grams of material in a Waring blender jar marked at the 300-ml. level.

3. Add 180 ml. of acetone and blend 2 minutes at high speed.

4. Dilute up to the 300-ml. mark with tap water.

5. Blend one additional minute.

6. Filter through 32-cm. Whatman No 2V fluted filter paper and collect 150 ml. of filtrate in a graduated cylinder.

7. Transfer the filtrate to a 1000-ml. separatory funnel.

8. Add 150 ml. of chloroform and shake for 30 seconds.

9. Allow the phases to separate and drain the lower phase through Whatman No 2V fluted filter paper into a 1000-ml. round bottomed flask.

10. Repeat Steps 8-9 twice more with fresh 75-ml. portions of chloroform.

11. Evaporate the combined extracts just to dryness on a rotary vacuum evaporator at 40°C. Proceed to Oxidation.

Extraction Procedure - (Continued)

Peanut Meat

1. Place 100 grams of whole peanuts into a blender jar and blend dry until sample is finely granular.

2. Transfer a 25-gram sub-sample to a clean blender jar. Add 10 grams of Hyflo Super-cel and 200 ml of acetone and blend for two minutes at high speed.

3. Filter with vacuum through Whatman No 42 filter paper in a Size 2A Büchner funnel.

4. Transfer the filtrate to a 500-ml. separatory funnel.

5. Reblend the filter cake with 200 ml. of chloroform and filter as before.

6. Transfer the filtrate to the separatory funnel containing the acetone extract.

7. Shake the separatory funnel for 30 seconds. Allow the phases to separate and drain the lower phase through Whatman No 2V, 32 cm. fluted filter paper containing a teaspoonful of Hyflo Super-cel, into a 1000-ml. round bottomed flask. Rinse the filter paper with a fresh 25-ml. portion of chloroform.

8. Evaporate the filtrate just to dryness on a rotary vacuum evaporator.

9. Transfer the residue to a 500-ml. separatory funnel using 250 ml. of Skellysolve B. (Use Skellysolve B which has been previously saturated with acetonitrile).

10. Rinse the flask with 150 ml. of acetonitrile and add to the separatory funnel. (Use acetonitrile which has been previously saturated with Skellysolve B).

11. Shake the separatory funnel for 30 seconds, allow the phases to separate and drain the lower phase into a second 500-ml. separatory funnel containing 100 ml. of Skellysolve B (Use Skellysolve B which has been previously saturated with acetonitrile).

12. Shake the second separatory funnel for 30 seconds, allow the phases to separate and drain the lower phase into a 500-ml. round bottomed flask.

13. Repeat Steps 10-12 with a fresh 100-ml. portion of acetonitrile.

14. Evaporate the combined acetonitrile extracts just to dryness on a rotary vacuum evaporator at 40°C. Proceed to Oxidation.

Cured tobacco, peanut hulls, peanut vines, pineapple bran, pineapple forage

1. Grind dry samples in a Wiley mill so that they will pass a No. 3 screen. Grind wet samples in a Hobart food chopper with an equal weight of dry ice. Place in frozen storage overnight to allow the dry ice to sublime.

Extraction Procedure - (Continued)

2. Weigh a 25-gm. portion into a blender jar.
3. Add 300 ml. of 60:40 methanol:H_2SO_4 (0.05N)
4. Blend for two minutes at high speed.
5. Filter with vacuum through Whatman No 541 filter paper, covered with a 1/4" layer of Super-cel, in a size 2A Büchner funnel.
6. Rinse the blender with 100 ml. of extraction solvent mixture and use this to wash the filter cake.
7. Transfer the filtrate to a 1 liter separatory funnel.
8. Rinse the filter flask with 200 ml. of chloroform and add this to the separatory funnel.
9. Shake the separatory funnel for 30 seconds.
10. Allow the phases to separate and drain the lower phase into a 1 liter round bottomed flask.
11. Repeat the chloroform extraction steps twice more. Use a 200 then a 100-ml. portion of fresh chloroform.
12. Evaporate the combined chloroform extracts to near dryness on a rotary vacuum evaporator (about 2 ml. of water will remain). Proceed to Aqueous Wash Steps.

Aqueous Wash

1. Add 200 ml. of chloroform to the residue from initial extraction and transfer to a 500-ml. separatory funnel.
2. Rinse the flask with 200 ml. of 0.05N H_2SO_4 and add this to the separatory funnel.
3. Shake the separatory funnel 30 seconds.
4. Allow the phases to separate and drain the lower, chloroform phase into a 500-ml. round bottomed flask.
5. Add 100 ml. of fresh chloroform to the separatory funnel and repeat the extraction.
6. Evaporate the combined extracts just to dryness on a rotary vacuum evaporator.

Oxidation

(Start a 5% standard at this point)

1. Dissolve the residue from the aqueous wash steps in 2 ml. of acetone.
2. Add 5 ml. of 20% $MgSO_4$ solution and 20 ml. of 0.1M $KMnO_4$ solution, washing down the sides of the flask during the additions.
3. Mix and let stand for 15 minutes.
4. Transfer to a 125-ml. separatory funnel using 20 ml. of chloroform to complete the transfer.
5. Shake the separatory funnel for 30 seconds, allow the phases to separate (centrifuge if necessary), and drain the lower phase through Whatman No 541 filter paper containing a teaspoonful of powdered sodium sulfate, into a 300-ml. round bottomed flask.
6. Repeat the chloroform extraction two additional times with fresh 20-ml. portions of chloroform.
7. After the final extraction, wash the sodium sulfate and filter paper with about 10 ml. of chloroform.
8. Evaporate the combined extracts just to dryness on a rotary vacuum evaporator at 40°C.

Florisil Column
(Tobacco and peanut hulls only)

1. Tamp a plug of glass wool into the bottom of a 20 x 400 mm. chromatographic column.
2. Fill the column up to the reservoir with 20% acetone in chloroform.
3. Slowly sprinkle in 7 gms. of 7% water deactivated Florisil and allow to settle.
4. Top the column with about one inch of granular sodium sulfate.
5. Drain the solvent down to the top of the sodium sulfate.
6. Dissolve the residue from the oxidation steps in 5 ml. of 20% acetone in chloroform and transfer to the column.
7. Rinse the flask with three 2-ml. portions of 20% acetone in chloroform and add each to the column just as the previous rinse has drained into the sodium sulfate layer.

8. Finally, elute the column with 90 ml. of 20% acetone in chloroform at a rate of 2-3 drops per second and collect the total eluate in a 250 ml. round bottomed flask.

9. Evaporate the eluate just to dryness on a rotary vacuum evaporator at 40°C.

Gas Chromatographic Analysis

Modification of the Flame Detector - Place 0.3 gram of finely powdered analytical reagent potassium chloride in a Parr press with a 1/4 inch diameter chamber and press into a solid pellet. (This produces a pellet approximately 1/4 inch in diameter and 1/4 inch long). Drill a hole half-way through the pellet lengthwise, the same as the flame jet diameter. Complete the through hole with a 1/32 inch drill bit. Place the pellet firmly on the jet tip. Position the collector electrode approximately 1 mm. above the pellet.

Column:	1 foot x 4 mm i.d. borosilicate glass column packed with 6% QF-1 solution coated on 80 - 100 mesh Gas Chrom Q.
Carrier gas:	Helium, 100 ml. per minute
Air flow:	425 ml. per minute
Hydrogen flow:	Adjust hydrogen flow so that approximately a 1/2 full scale peak results from a 5 nanogram injection.
Temperatures:	Column 230°C. Injection port 225°C. Detector 240°C.
Recorder chart speed:	1/2 inch per minute

1. Dissolve the residue from the previous steps in 4 ml. of acetone (Samples containing 25-gram equivalents of extract should be dissolved in 2 ml. of acetone).

2. Inject 4 microliters of the sample or standard solution into the gas chromatograph.

3. Identify the BAY 68138 sulfone peak by its retention time and measure the area produced on the recorder strip chart with a polar planimeter. At the operating conditions employed, the retention time for BAY 68138 sulfone is approximately 4.5 minutes.

Calculations

Calculation of the ppm. of BAY 68138 in a sample is done by use of the following equation in which the response for an unknown is compared to the response for a known amount of BAY 68138 standard carried through the procedure from the oxidation step.

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$$ppm = \frac{\text{(Sample) Area}}{\text{(Standard) Area}} \times \frac{\text{(Sample) Attenuation}}{\text{(Standard) Attenuation}} \times \frac{\text{ng. std. inj.}}{\text{spl. wt. in gms.}} \times \frac{\text{final vol. (ml.)}}{\mu\text{l. inj.}}$$

For samples which are split after the initial extraction step, the "sample weight" term in the above calculations must be divided by 2.

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TABLE I

Recovery of BAY 68138 and Metabolites from Various Crops[a]

Crop	Compound Added	Ppm. Added	% Recovery
Orange Peel	Control	-	0
" "	BAY 68138	0.1	89
" "	BAY 68138 Sulfoxide	0.1	125
" "	BAY 68138 Sulfone	0.1	110
Orange Pulp	Control	-	0
" "	BAY 68138 Sulfoxide	0.1	79
" "	BAY 68138 Sulfone	0.1	94
Peanut Hulls	Control	-	0
" "	BAY 68138	0.1	71
" "	BAY 68138 Sulfone	0.1	89
Peanut Meat	Control	-	0
" "	BAY 68138	0.1	96
" "	BAY 68138 Sulfoxide	0.1	96
" "	BAY 68138 Sulfone	0.1	79
Peanut Vines	Control	-	0
" "	BAY 68138	0.5	84
" "	BAY 68138 Sulfoxide	0.5	97
Pineapple Bran	Control	-	0
" "	BAY 68138	0.1	87
" "	BAY 68138 Sulfone	0.1	108
Pineapple Forage	Control	-	0
" "	BAY 68138	0.1	76
" "	BAY 68138 Sulfoxide	0.1	79
" "	BAY 68138 Sulfone	0.1	82
Pineapple Fruit	Control	-	0
" "	BAY 68138	0.1	95
" "	BAY 68138 Sulfoxide	0.1	91
" "	BAY 68138 Sulfone	0.1	94
Tobacco (Dry)	Control	-	0
" "	BAY 68138	0.5	78
" "	BAY 68138 Sulfoxide	0.5	88
" "	BAY 68138 Sulfone	0.5	88

(a) Raw data for the above recoveries are included in Chemagro Report No. 25,542.

Discussion

The method described in this report measures not only BAY 68138 residues, but also BAY 68138 sulfoxide and sulfone. Although all three of these compounds give a peak when injected into a gas chromatograph, simplicity and sensitivity considerations make it more desirable to oxidize the parent compound and the sulfoxide to the sulfone with subsequent gas chromatographic analysis of only one peak. Room temperature oxidation is quantitiative using 0.1 M potassium permanganate (4) for 15 minutes. Oxidation also converts most plant extractives and pigments to a water soluble form, making them easy to remove.

A Florisil column step is included for certain crops such as tobacco which require additional cleanup after oxidation, prior to gas chromatographic analysis. Crops, other than tobacco, may be run through the column step if necessary for further cleanup.

The solution coating technique (1) used to prepare the gas chromatographic column packing was necessary to produce a column without tailing or adsorption. In addition, the column was "no flow" conditioned overnight at 250°C for 2 hours followed by flow conditioning at operating conditions at least 4 hours before use. Several columns have been prepared with identical results indicating the procedure to be reproducible.

Recovery experiments were run on a number of different crops by adding known amounts of BAY 68138 and metabolites at the extraction step. In general, the recoveries were run at the 0.1-ppm. level. Where samples were fortified with metabolites other than BAY 68138, an appropriate amount of that compound was oxidized for use as a standard. Results are listed in Table I. Typical chromatograms for controls and recoveries of BAY 68138 from tobacco and peanuts are shown in Figures 1-5.

The efficiency of the extraction procedure was tested by fortifying large samples of tobacco with BAY 68138 and metabolites at the 0.5-ppm. level. Each sample was treated with a separate compound. These fortified samples were allowed to stand at room temperature for 24 hours prior to extraction and analysis. Recovery of the three compounds averaged 84%, the lowest of which was 78% for the parent compound, indicating the extraction to be adequate.

A standard curve was run to determine linearity of response in the gas chromatograph for the sulfone. Response was linear over a fifty-fold range up to at least 25 ppm. Any samples containing residues in excess of this figure should be diluted and re-injected to insure that response falls along the linear portion of the curve.

If 0.1 square inch is considered the smallest area which can be accurately measured with a polar planimeter, the level of sensitivity is determined by the amount of standard BAY 68138 necessary to produce this area. In general, 0.1 ppm. of standard produced a peak of 1 square inch or better indicating the sensitivity of the method to be approximately 0.01 ppm. It should be emphasized that due to changes in sensitivity from day to day and from one instrument to another, this limit of sensitivity will vary. Also, the size of any control peak will vary with the instrument sensitivity.

Discussion - (Continued)

To determine the specificity of the method for BAY 68138, an interference study was conducted. Because of the specificity of the thermionic detector employed, only organophosphorus compounds were tested as possible interferences. All of the organophosphate chemicals registered for use on citrus, peanuts and pineapples were run at the highest registered level as listed in the N.A.C. Bulletin (2). For tobacco, all phosphorus containing pesticides listed in the 1968 edition of Handbook No. 331 (3) for use on tobacco, were run at the 1 ppm. level. A list of these compounds and the level at which they were tested is shown as Table II. No interferences were noted from any of these compounds. Raw data and chromatograms for the interference study are shown in Chemagro Report No. 25,533.

A confirmatory method for BAY 68138 and metabolites employs the identical sample preparation procedure as listed above however gas chromatographic analysis employs a column of different polarity. The confirmatory column is a 1 foot x 4 mm. i.d. borosilicate glass tube packed with 5% OV-17 solution coated (1) on 80-100 mesh Gas Chrom Q. The column temperature is 240°C with a helium carrier gas flow of 100 ml. per minute. Other conditions are the same as in the original procedure. Retention time with the confirmatory column at these conditions is approximately 5.0 minutes. Raw data and chromatograms for the confirmatory method are shown in Chemagro Report No. 25,534.

TABLE II

Compounds Tested For Interference[a] With The BAY 68138 Cron Method

Compound	Ppm. Level Tested
Delnav	2.8 ppm.
Diazinon	1 ppm.
DI-SYSTON	1 ppm.
Dyfonate	1 ppm.
EPN	3 ppm.
Ethion	2 ppm.
Ethyl Parathion	1 ppm.
GUTHION	1 ppm.
Malathion	8 ppm.
Methyl Parathion	1 ppm.
Naled	3 ppm.
Phorate	0.1 ppm.
Phosdrin	0.25 ppm.
Phosphamidon	0.75 ppm.
SYSTOX	0.75 ppm.
Trichlorfon	1 ppm.
Trithion	2 ppm.

[a]No interferences were noted from any of the above listed chemicals.

TABLE III

List of Figures

Figure 1	Peanut Meat Control
Figure 2	Peanut Meat Control + 0.1 ppm. BAY 68138
Figure 3	Tobacco Control
Figure 4	Tobacco Control + 0.5 ppm. BAY 68138 sulfone
Figure 5	Typical 0.1 ppm. BAY 68138 standard

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Sample & No. #365 Date 7/22/69

Pesw = Control

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Areas: Sample 0.05 Sq. In., Standard 1.51 Sq. In.

BR 69-49-66 Chrom. No. 8912

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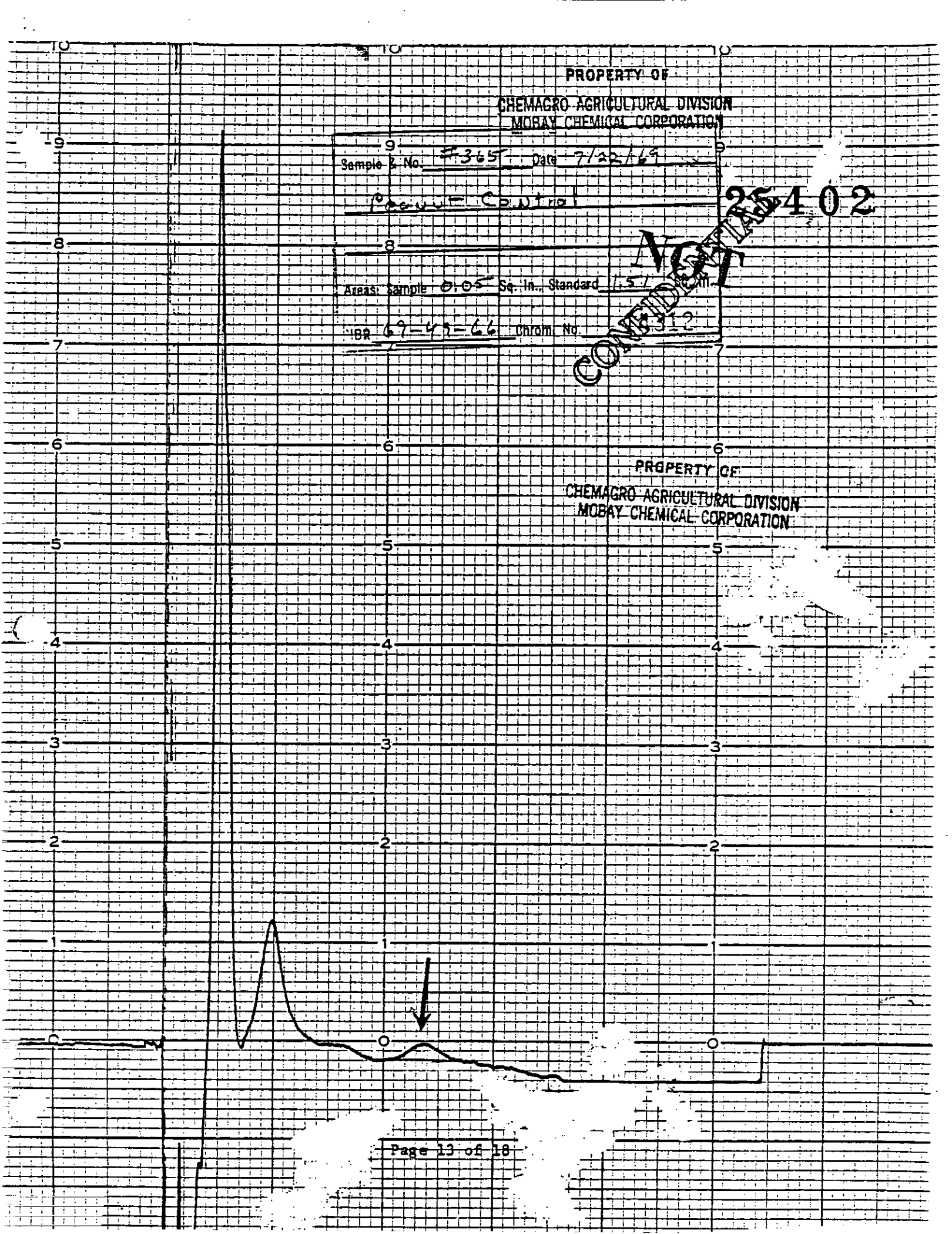

Sample & No. H 365 Date 7/22/65

Fenoxycarb 10.1 ppm

Bay 68138

Areas: Sample 1.49 Sq. In., Standard 1.51 Sq. In.

NBR 69-49-66 Chrom. No. 14

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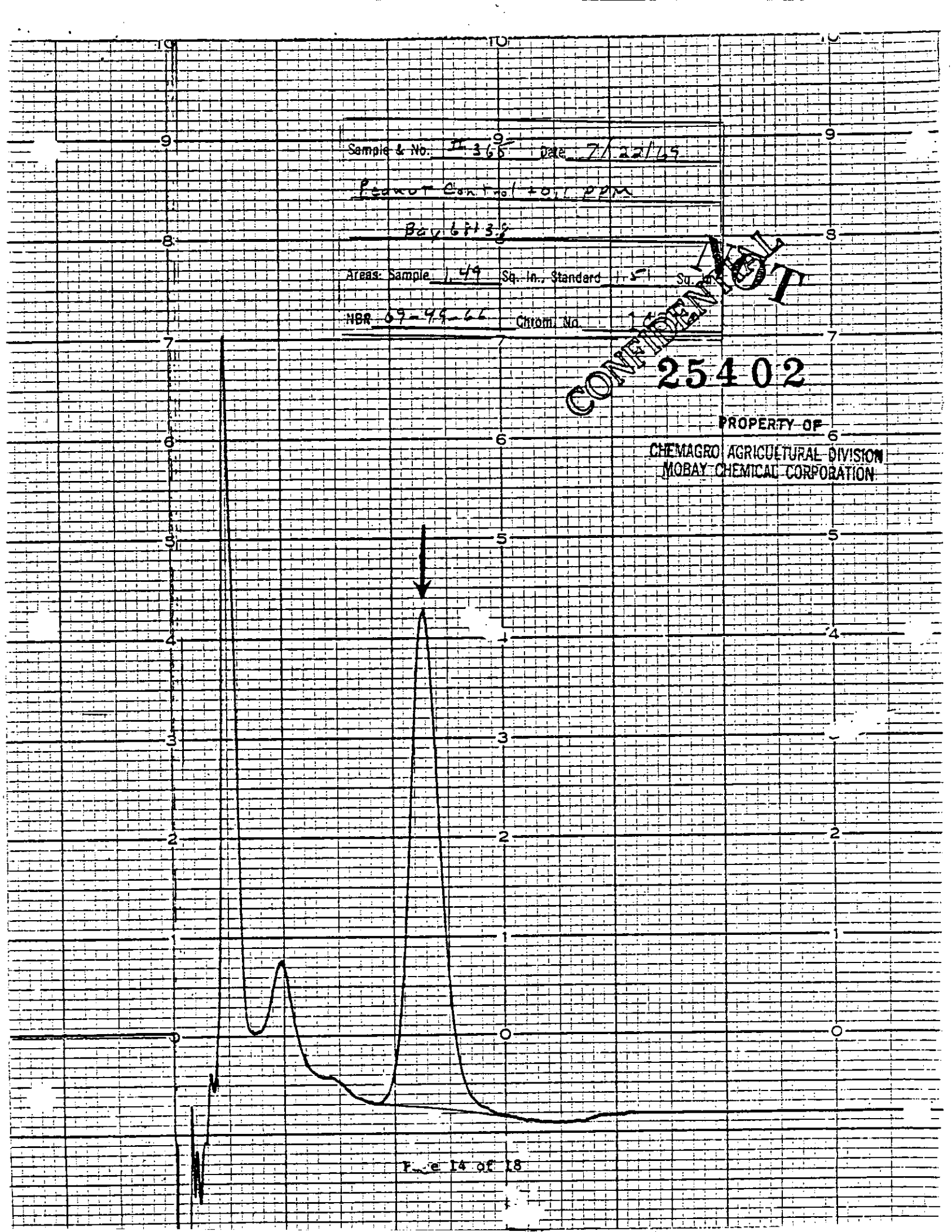

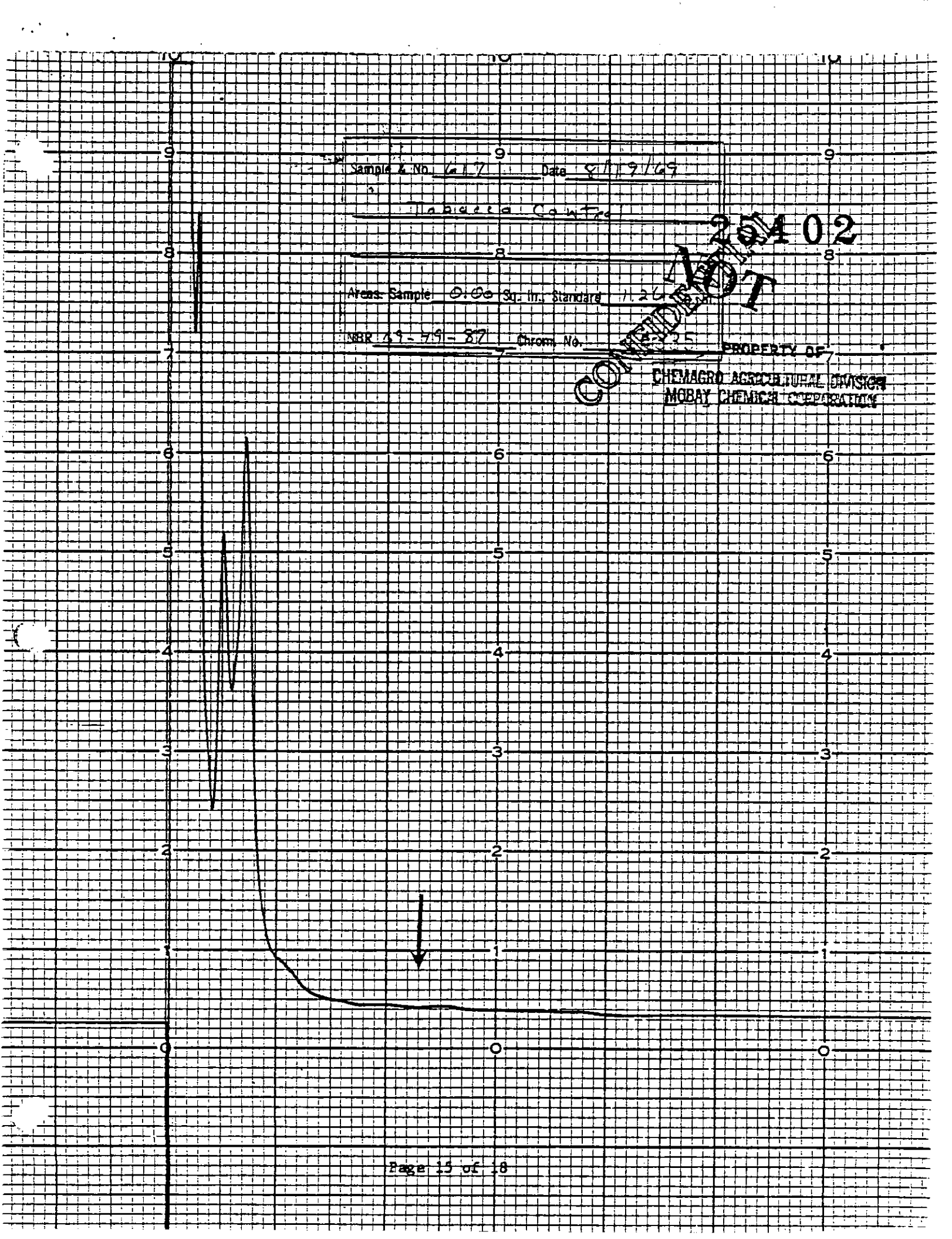
Sample & No. 617 Date 8/19/69
Tabacco Cont.
Areas: Sample 0.00 Sq. In., Standard 1.26 Sq. In.
NBR 49-79-87 Chrom. No. 4-325
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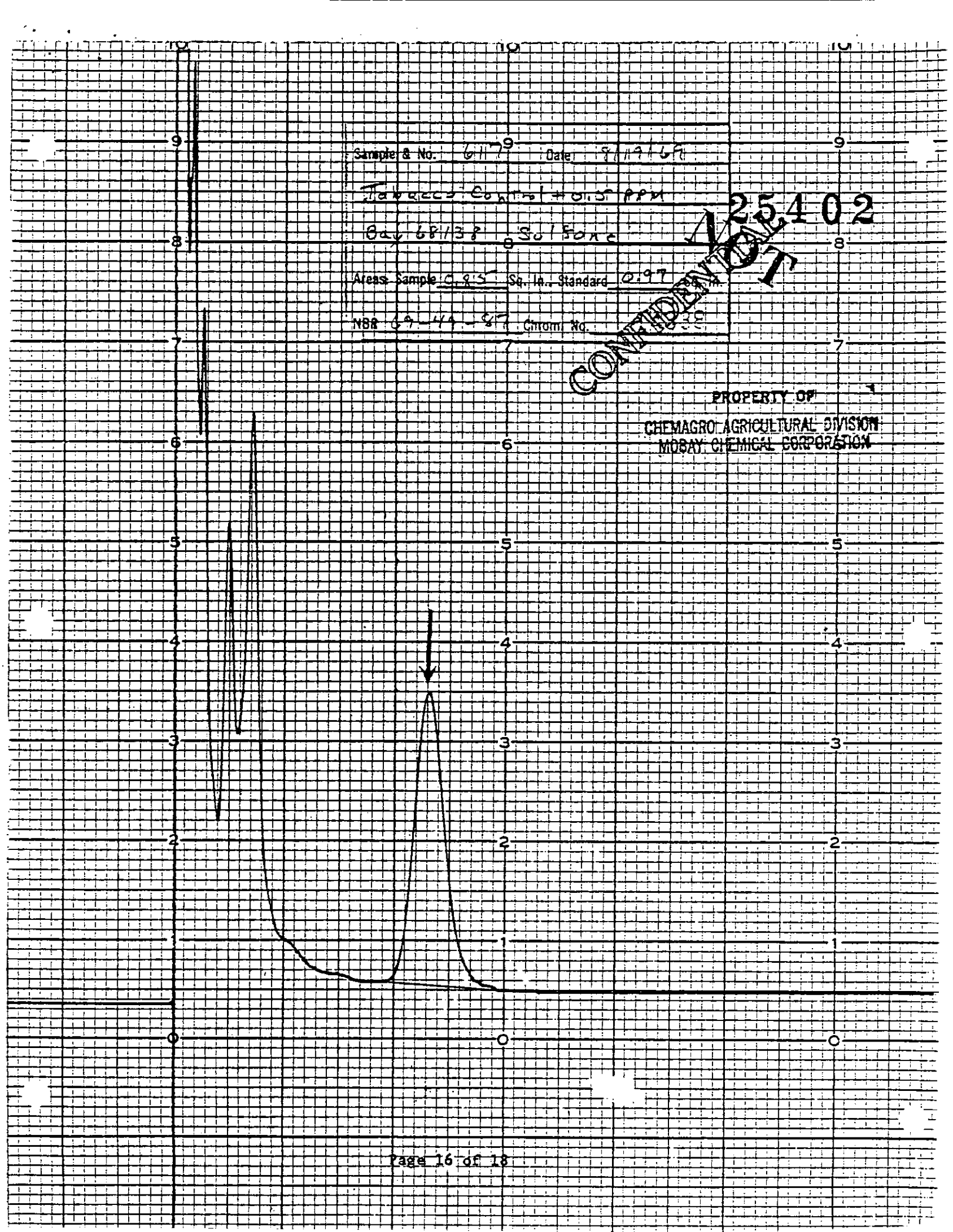
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Tordon Control +0.5 PPM
Bay 68138 Soil Core
Areas: Sample 0.85 Sq. In., Standard 0.97 Sq. In.
N88 64-44-87 Chrom. No.
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Sample & No. [illegible] Date 11-22-69

Bay 6238 Standard

0.1 PPM

Areas: Sample _____ Sq. In.; Standard 1.3 Sq. In.

YBR 69-45-46 Chrom. No. [illegible]

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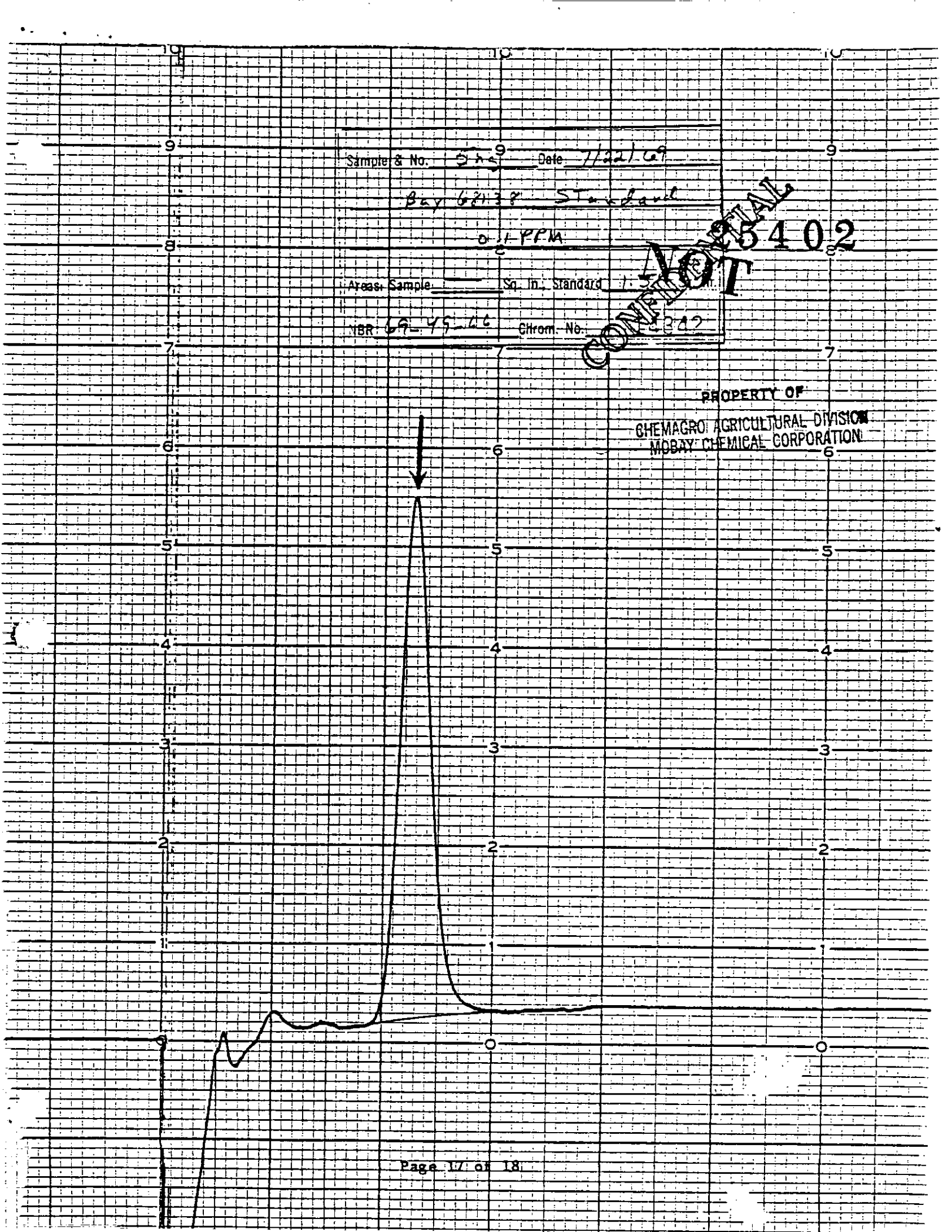

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(2) N.A.C. News and Pesticide Review, vol. 27, No 3 (1969).

(3) Suggested Guide for the Use of Insecticides to Control Insects Affecting Crops, Livestock, Households, Stored Products, Forest, and Forest Products - 1968, Agriculture Handbook No. 331, U.S. Department of Agriculture.

(4) Tietz, H., Frehse, H., **Höfchen-Briefe 13, 212-21 (1960).**

(5) Waggoner, T. B., Metabolism of Ethyl 4-(methylthio)-m-tolyl isopropylphosphoramidate (BAY 68138) in Plants. Chemagro Report No. 25,519 (1969).